Nomenclature of the Proteins of Cow's Milk: Third Revision

DYSON ROSE, Chairman
Division of Biology, National Research Council of Canada, Ottawa
J. R. BRUNNER
Department of Food Science, Michigan State University, East Lansing 48823
E. B. KALAN
Milk Properties Laboratory, Eastern Utilization and Development Division
USDA, Philadelphia, Pennsylvania 19118
B. L. LARSON
Department of Dairy Science, University of Illinois, Urbana 61801
P. MELNYCHYN
Carnation Company Research Laboratory, Van Nuys, California 91401
H. E. SWAISGOOD
Department of Food Science, North Carolina State University, Raleigh 27607
and
D. F. WAUGH
Department of Biology, Massachusetts Institute of Technology, Cambridge 02139

Abstract

This report reviews the changes and additions to the nomenclature of the major casein fractions, and of β-lactaglobulin, that have been necessary over the past five years. In addition, fairly extensive reports are included on γ-casein, α-lactalbumin, the immune globulins, and the proteose-peptone fraction. The information is summarized in tabular form.

The nomenclature of all milk proteins remains fluid and there seems little likelihood that the situation will stabilize, i.e., that discovery of additional components and variants will cease.

Introduction

As noted in the Committee's previous report (126), the selection of specific, definitive nomenclature systems has become more and more difficult as our knowledge of the complexity of the milk proteins has increased. We have, for example, been forced to add superscript numbers (65, 101, 102) and a subscript letter (124) in the nomenclature of β-casein, and there is reason to assume that more variants will be discovered. Most variants discovered to date can be separated by zonal electrophoresis, and the nomenclature is related to their mobility. However, β-casein B_Z (7, 124) is one exception and it is probable that other variants differing in uncharged amino fats will be discovered; indeed, it has been suggested (100) that differences in amino acid analyses reported by different laboratories reflect such genetic variations. Under these conditions, the nomenclature necessarily reflects the incomplete state of our knowledge, and your Committee can only review and comment without suggesting a uniform procedure for selecting designations.

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Caseins

α_s-Caseins. The situation with regard to α_{s1}-casein is essentially unchanged since the previous revision (126), except that a newly discovered variant, α_{s1}-D, must be included. This variant has a mobility greater than B but less than A (R_m = 1.13 on polyacrylamide gels, pH 9.1, 4.5 M urea). It has been reported as occurring in French Flamande (44) and certain Polish (87) breeds. An excellent review on the breed distribution of milk protein variants has been published by Aschaffenburg (6).

The amino acid composition of the four known variants of the α_{s1}-series is shown in Table 2. While agreement among these values is excellent, it should be assumed that corrections will be necessary. The molecular weight (28,600) selected for the calculation may be high (114). It is also of interest to note that an α_{s1}-casein prepared by a novel method (84) was reported to contain 20.5 residues of aspartic acid and 50.2 of glutamic acid per 28,600. Further work on caseins prepared by novel methods should be encouraged. Use of freshly prepared caseins is recommended; firm data are not available but the stability of purified preparations has been questioned.

There has been little increase in our knowledge of the other α_s- or α_s-like caseins, largely because of the extreme difficulty of purifying

TABLE 1. Proteins of cows' milk and some of their properties.[a]

Contemporary nomenclature	Approx % of skimmilk protein	Occurrence in electrophoretic pattern (Peak number)[b]	Electrophoretic mobility[c]	pI[d]	Sedimentation constant (S_{20})[e]	Molecular weight[f]	Components
α_s-Casein	45 to 55	1[g]	−6.7[g](61)	4.1(61)	3.99(121)	23,000(114)	α_{s1}-variants A, B, C, D. α_{s2}-, α_{s3}-
κ-Casein	8 to 15	1[g]	−6.7[g](61)	4.1(122)	1.4 (122)	19,000[l](132)	Variants A and B, subvariants containing 0 to 5 carbohydrate chains
β-Casein	25 to 35	2	−3.1 (61)	4.5(61)	1.57(121)	24,100(121)	Variants A^1, A^2, A^3, B, C, D, B_Z
γ-Casein	3 to 7	3	−2.0 (61)	5.8 to 6.0(61)	1.55(94)	30,650(94)	Variants A^1, A^2, A^3, B, Components R, S, and TS (TS has two variants)
α-Lactalbumin	2 to 5	4	−4.2 (41)	5.1(70)	1.75(41)	14,437(14)	Variants A, B in Zebu
β-Lactoglobulin	7 to 12	6	−5.3	5.3	2.7 (128)	36,000(128)	Variants A, A_{Dr}, B, B_{Dr}, C, D
Blood serum albumin	0.7 to 1.3	7	−6.7 (107)	4.7(107)	4.0 (34)	69,000(107)	
IgG Immunoglobulins							
IgG1	1 to 2	1 and 2	−2.0 to 2.2[j]		6.3[k]	150,000 to	A1 and A2 allotypes recognized on
IgG2	≃0.2 to 0.5	1	−1.1 (116)		6.6[k]	170,000[l]	serum IgG[l]
IgM Immunoglobulin	≃0.1 to 0.2	2	.....		18 to 19[k]	900,000 to 1,000,000[l]	Insufficient data
IgA Immunoglobulin	≃0.05 to 0.10	2(?)	.....		10 to 12[k]	300,000 to 500,000[l]	Insufficient data
Proteose-peptone fraction	2 to 6	3, 5, 8	−3.8 to 9.3[h]	3.3 to 3.7[h]	0.8 to 4.0[h]	4,100 to 200,000[h]	Multiple, including glycoproteins

[a] The values included are not necessarily the best available, and their inclusion in this table does not constitute endorsement by the Committee.

[b] Free-boundary electrophoresis, veronal buffer pH 8.6; casein components designated in descending order of mobility in casein pattern; whey proteins, designated in ascending order of mobility in acid whey pattern (77).

[c] Electrophoretic mobility = $\times 10^{-5}$ cm^2 volts^{-1} sec^{-1} in Tiselius moving boundary method, 2 C, veronal buffer, pH 8.6, $\Gamma/2$ 0.01; descending pattern.
[d] Isoelectric point, or pH of no electrophoretic migration.
[e] Sedimentation coefficient, $S_{20} = (dx/dt\ X\omega^2x)$ in Svedberg units ($5 = 1 \times 10^{-13}$) corrected to 20 C.
[f] Refer to original literature for methods and conditions.
[g] Value for whole α-casein (i.e., α- and κ-casein complex).
[h] Values taken from appropriate section of this revision.
[i] This value refers to the carbohydrate-free (A_o or B_o) fraction. Approximately 600 should be added for each carbohydrate chain.
[j] Average of values from Smith (116) and Murthy and Whitney (94). T-globulin and pseudoglobulin are considered to be primarily IgG1. Euglobulin is too heterogeneous to fit into the current nomenclature.
[k] Average of values available in the more recent literature.
[l] Average of more recent literature values and estimations made from reported sedimentation studies.

adequate quantities for study. Annan and Manson (2) have recently isolated an α_{s0} casein, which is similar to α_{s1}-casein, and a further fraction containing at least three ($\alpha_{s2,3,4}$-caseins) components.

β-Caseins. Our recognition of the complexity of the β-caseins has increased considerably over the past five years. β-Casein A has been demonstrated to exist in three genetically determined forms (65, 102), and the presence of two of these has also been observed in the milks of African and Indian Zebu cattle (7). The amino acid composition of a new variant, β-casein D, has also been reported (125).

The suggested nomenclature of the A variants as A^1, A^2, and A^3 (65) appears satisfactory, as does that of the D variant. However, the B_Z variant reported by Aschaffenburg, Sen, and Thompson (7) must be regarded as a special case, as it was detected by means of "fingerprints" of chymotryptic peptides and apparently cannot be distinguished from the B variant by electrophoretic methods. It thus represents the first example of the nomenclature difficulties discussed in our Introduction. The use of letters and superscript numbers for variants differing in electrophoretic mobility, and of letters and subscript letters for variants that do not differ in mobility, is a possible system of nomenclature, but it would necessitate designation of the more common B-variant of Western cattle with a subscript letter ($_W$?). Our Committee prefers to suggest that the B_Z designation be regarded as tentative and not as a precedent.

Publication in tabular form of the amino acid composition of the β-casein variants as absolute analyses would appear to be unjustified at this time, because analytical agreement among different laboratories is not fully satisfactory. It also appears that assignment of mRNA coding triplets at this time is unwarranted; the variants apparently differ by more than one amino acid.

κ-Casein. Our understanding of the complexity of κ-casein has increased considerably during the past five years. The two genetic variants previously reported (95, 112, 131) have been shown to differ by single residues of aspartic acid, alanine, threonine, and isoleucine (113, 132). Gel electrophoretic patterns and partial separation by chromatography on DEAE cellulose columns showed that each genetic variant consisted of several components (80, 109, 113, 132). Early attempts to isolate these components were only partially success-

TABLE 2. Amino acid composition of the genetic variants of α_{s1} casein. Residues amino acid per 28,600 molecular weight.[a]

Amino Acid	α_{s1}-Variant					
	A[b]	B[b]	B[c]	C[b]	C[c]	D[d]
Aspartic Acid	16.8	18.1	17.9	18.2	17.6	18.0
Threonine	6.7	6.0	5.7	6.1	5.8	6.3
Serine	17.8	17.3	16.8	17.6	16.6	15.8
Glutamic Acid	46.6	46.4	47.0	45.5	45.9	47.0
Proline	20.6	20.3	19.5	20.4	20.0	20.5
Glycine	10.7	10.7	10.7	11.8	11.8	10.9
Alanine	9.9	10.8	11.0	10.8	11.0	10.4
Valine	11.9	13.4	13.2	13.6	13.4	13.1
Methionine	5.9	5.7	5.7	5.7	5.6	5.3
Isoleucine	13.6	13.1	12.9	13.3	13.0	13.0
Leucine	17.3	20.3	20.1	20.5	20.1	20.1
Tyrosine	12.1	11.6	11.4	11.7	11.4	11.2
Phenylalanine	7.5	9.6	9.4	9.7	9.4	9.6
Tryptophan	2.8[e]	2.7[e]	3.4	2.8[e]	3.5	3.2
Lysine	18.1	17.0	16.3	17.0	16.2	16.6
Histidine	6.2	6.1	6.0	6.1	6.0	5.8
Arginine	6.2	7.2	7.0	7.2	7.0	7.2
NH_3	27.1	31.1	32.3	29.7	33.1	33.0
PO_3H	11.3	11.3	11.5	11.3	11.7	11.4

[a] α_{s1} -A calculated on the basis of 28,000 molecular weight.
[b] Gordon et al., 1965 (40).
[c] de Koning and van Rooijen, 1965 (72).
[d] de Koning and van Rooijen, 1967 (73).
[e] More recent analyses indicate this value to be 2.0 residues.

TABLE 3. Amino acid composition of κ-casein, *para*-κ-casein, and the macropeptide.

	Para-κ-casein		Macropeptide		κ-Casein	
			A	B	A	B
Reference	(67)	(63)	(74)	(74)	(132)	(132)
Aspartic Acid	7	8.2	4.3	3.5	12	11
Threonine	3	5.2	9.8	8.8	14	13
Serine	7	7.6	4.7	4.8	12 to 13	12 to 13
Glutamic Acid	18	17.9	8.8	8.7	27	27
Proline	13	12.9	6.6	6.4	20	20
Glycine	1	1.7	1.1	1.1	3	3
Alanine	9	9.7	4.2	4.9	13 to 14	14
Valine	5	6.5	4.9	4.9	10 to 11	11
Cystine/2	2	1.8			2	2
Methionine	1	1.3	0.7	0.7	2	2
Isoleucine	6	7.4	4.9	5.7	11	12
Leucine	7	8.0	1.0	1.0	8	8
Tyrosine	9	8.8			8	8
Phenylalanine	4	4.1			4	4
Lysine	7	7.0	2.8	2.9	9	9
Histidine	3	2.9			3	3
Arginine	5	5.0			5	5
Tryptophan	1[a]	1[a]			1[a]	1[a]
Total residues	108	116				

[a] Value reported by Spies (Anal. Chem., 39: 1412. 1967) for κ-casein.

ful, but showed that they differed in carbohydrate content (80, 113, 132).

Studies on *para*-κ-casein have helped to resolve this complexity. Several authors reported (36, 63, 79, 132, 134) that *para*-κ-casein was heterogeneous, even when prepared with crystalline rennin[1] from a single genetic variant of κ-casein. MacKinlay et al. (79) were also able to show that the minor (slowest moving in gels above pH 7.5) component of the *para*-κ-casein arose from some of the minor fractions of κ-casein, whereas the major *para*-κ-casein was formed from the major, carbohydrate-free component of κ-casein. Kim et al. (67) clarified this situation by showing that rennin treatment of κ-casein preparations that had not been exposed to urea (or certain other reagents) formed only the major *para*-κ-casein component. All minor *para*-κ-casein components appear to be artifacts formed by modification of the net charge of the κ-casein or *para*-κ-casein molecule. The commonest cause of the change in net charge is the conversion of lysine to homocittruline by cyanate present in urea solutions, but similar changes in mobility can be induced by exposure to guanidine hydrochloride (134) and probably by some alkylation procedures.

Elimination of these artifacts reduced the number of components of a genetic variant of κ-casein to about six. These probably differ only in the number of attached carbohydrate chains, which vary from zero in the major component to five in the most minor component detected to date (133). The use of subscript numbers to designate the number of carbohydrate chains (e.g., κ-casein A_0, A_1, etc.) has been suggested (133).

Table 3 presents selected amino acid analyses for κ-casein and the two components obtained by primary rennin action.

Para-κ-casein. Two signficant advances have occurred that affect the nomenclature of *para*-κ-casein. The first is the recognition that the

[1] The term, rennin, designates a purified enzyme obtained from rennet, an extract of calves' stomach. We suggest that use of rennin to describe bacterial or mold enzymes having milk-clotting activity should be discouraged.

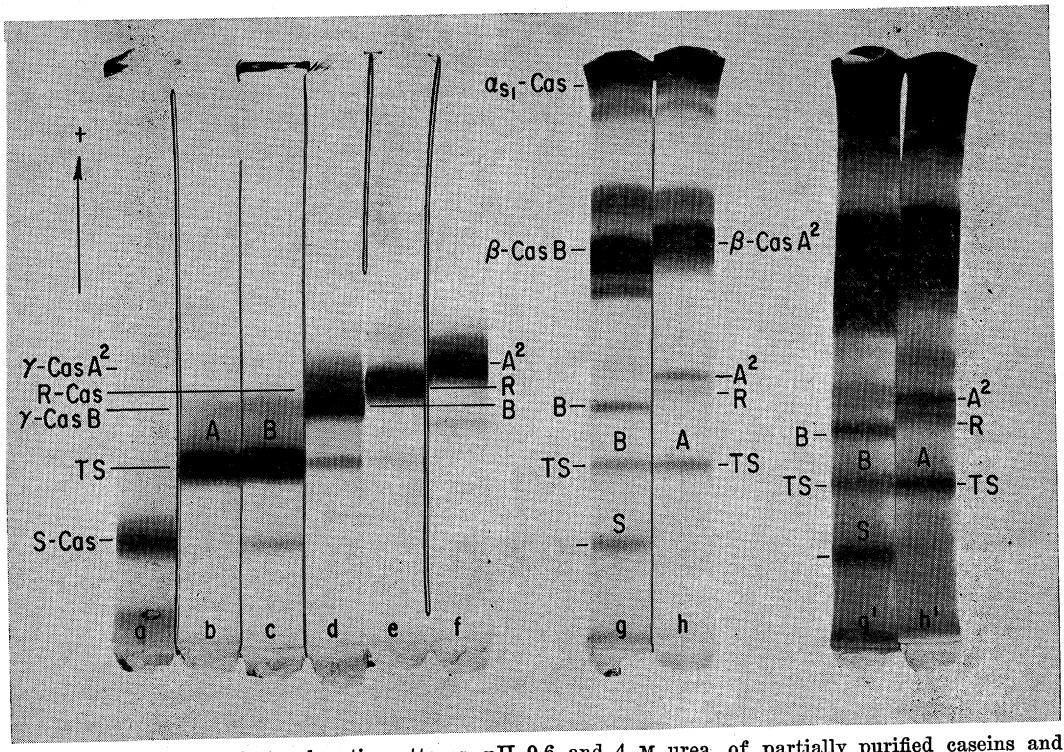

FIG. 1. Disc gel electrophoretic patterns, pH 9.6 and 4 M urea, of partially purified caseins and of the original caseins, Types B and A^2 with respect to β-casein. Slots *a* through *f* are purified caseins, with γa^2 prepared from milk typed β-A^2 and γB prepared from milk typed β-B. a = S-Casein. b = TS-Casein A. c = TS-Casein B. d = γ-Casein B. e = R-Casein. f = γ-Casein A^2. g = Unfractionated casein typed β-, γ-casein B. h = Unfractionated casein typed β-, γ-casein A^2. g^1 = Same as *g*, only twice the concentration of casein. h^1 = Same as *h*, only twice the concentration of casein.

minor components reported by several authors are artifacts (*cf.* above).[2] The second is that the amino acid differences between variants A and B both occur in the peptide portion that is released by rennin (67, 74). Thus, *para*-κ-casein appears to be a single invariant protein regardless of the κ-casein component from which it is formed.

The soluble peptide released by rennin is, of course, a multicomponent fraction (1, 4) carrying both the genetic variations and the variable amount of carbohydrate. The obvious procedure for designating specific components of the macropeptide will be by identifying the κ-casein from which they were released. Because a major component of the soluble peptide from unfractionated κ-casein will not contain carbohydrate, we recommend disuse of the term glyco for such peptides.

γ-Casein. Mellander (83) demonstrated the existence of three electrophoretic fractions of casein which he named α-, β-, and γ-casein in order of decreasing mobility, and the first Committee Report [Jenness et al. (61)] accepted γ-casein as one of the three principal components of casein. Hipp et al. (58, 59) devised methods for separating γ-casein, by fractionation with 50% ethanol and with urea, and obtained preparations that were electrophoretically homogeneous on the alkaline side of the isoelectric point, pH 5.8 to 6.0, but heterogeneous on the acid side. This γ-casein had a low phosphorus content, 0.11%, and it also had a high sulfur content, 1.03%, relative to α- and β-caseins. The preparation was thought to be similar to the alcohol-soluble, low-phosphorus casein isolated by Osborne and Wakeman (98).

The first revision (19) of the Committee's Report (61) revealed little or no progress in the elucidation of γ-casein. Murthy and Whitney (94) concluded that though γ-casein and immune globulins are different proteins, they are all present in the slow-moving peak in the electrophoretic pattern of skimmilk at pH 8.7. The molecular weight of γ-casein was given as 30,650 and found to be dependent on pH, buffer ion, and temperature. By 1960, more powerful tools were available and preparation of γ-casein by column chromatography was achieved by Groves et al. (50). With the method of Wake and Baldwin (130) for determining homogeneity in starch-gel-urea electrophoresis, Groves et al. (50) found that γ-casein was eluted from DEAE-cellulose columns with 0.02 M phosphate buffer, pH 8.3. Conditions of chromatography and electrophoresis were carefully determined and essentially homogeneous preparations were obtained.

The first indication of genetic polymorphism in the γ-casein fraction of bovine milk was noted by Aschaffenburg (5). Later, El-Negoumy (35) demonstrated polymorphism in the γ-casein fraction of milks of 44 cows and concluded that the γ-casein fraction consisted of five main components, γ_1 to γ_5, three of which occur as two variants designated A and B. Groves and Kiddy (48) used disc gel electrophoresis, pH 9.6 in 4 M urea, to reveal γ-casein polymorphism in milks of individual cows. These workers defined γ-casein as that fraction eluted at 0.02 M phosphate, pH 8.3, from DEAE-cellulose columns as described by Groves et al. (50). More recently, by employing column chromatography and gel electrophoresis in acid and alkaline media, Groves et al. (47, 49, 129), have revealed the existence of at least four polymorphic γ-casein proteins and at least three other minor proteins, designated R, S, and TS. In addition, they have shown an interesting relation between the β-casein polymorphs and the similarly designated γ-casein polymorphs. In this connection, it is of some interest to note that their γ-casein apears to be absent in milks found to be homozygous with respect to β-casein C. Further, the occurrence of the other minor proteins appears to be related to the appearance of specific β-, γ-casein polymorphs in the milks of individual cows.

To clarify the γ-casein nomenclature, disc and vertical gels are illustrated in Figures 1 and 2, respectively. The R protein occurs only when γ-casein A is present, the S protein only when γ-casein B is present. In milks heterozygous for γ-casein, both R and S proteins are found. It should be noted that the γ-casein A[2] is not resolved from the R protein in vertical gel electrophoresis in an alkaline medium. However, there is a satisfactory separation under similar conditions using disc electrophoresis.

[2] Hill and Wake (57) maintain that the second *para*-κ-casein is not an artifact, but they do so on the assumption that "Reaction with cyanate during the electrophoresis itself (in starch gel-urea) could not account for the appearance of a sharp band." This assumption is not valid, because the gel was heated in the presence of urea and sufficient cyanate is, therefore, present to react with casein before migration begins (29). Cyanate reacts rapidly with SH groups (120), and probably reacts rapidly with ε-amino groups; the slow reaction rate normally attributed to carbamylation results from the slow formation of cyanate at or below ambient temperatures.

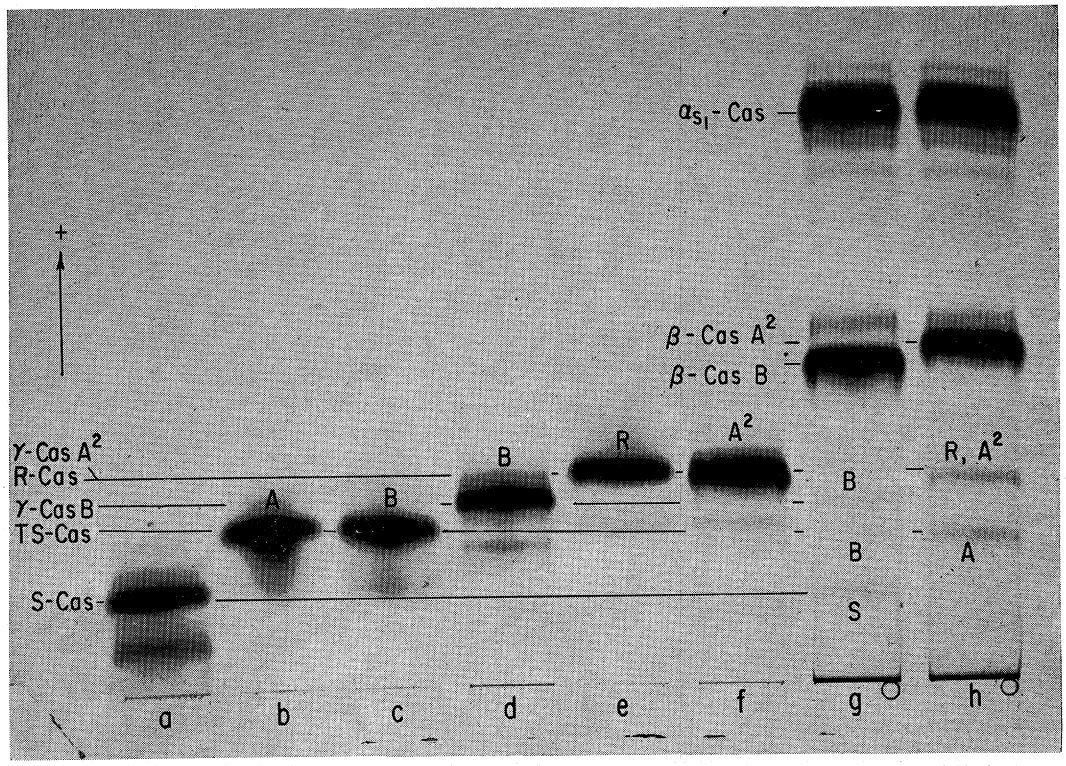

FIG. 2. Vertical gel electrophoresis, pH 8.6 and 4.5 M urea, of the same samples shown in Figure 1. a = S-Casein. b = TS-Casein A. c = TS-Casein B. d = γ-Casein B. e = R-Casein. f = γ-Casein A^2. g = Unfractionated casein typed β-, γ-casein B. h = Unfractionated casein typed β-, γ-casein A^2.

The TS protein, which is also polymorphic as determined by zone electrophoresis at acid pH, occurs in all milks. The relation between γ-casein and β-casein polymorphs may also be adduced from Figures 1 and 2. Gamma casein, typed A in alkaline media, can be shown to be polymorphic in acid media, three variants, A^1, A^2, and A^3, being distinguishable, with A^1 having the fastest mobility of the three A variants (in acid media, γ-casein B travels faster than A variants). The TS protein includes two genetic species, A and B, demonstrated in acid gel electrophoresis (A having faster mobility in acid).

Table 4 lists the amino acid composition of some γ-casein variants. These data include the earlier work of Gordon et al. (42) and the as yet unpublished data of Groves and Gordon (46).

In view of the above results, it seems necessary to clarify the nomenclature of γ-casein. Your Committee suggests that the definition given previously (126), i.e., "a fraction of whole casein soluble in 3.3 M urea but insoluble in 1.7 M urea at pH 4.7 upon the addition of $(NH_4)_2SO_4$" be maintained. However, in accord with the precedent of α-casein, this definition should now be considered as a generic name for "whole-γ," and a specific fraction of this complex, which is eluted from a DEAE column with 0.02 M phosphate buffer pH 8.3 under conditions specified by Groves et al. (50) may be termed γ-casein. Thus, the R, S, and TS proteins may be considered as parts of the whole-γ but distinct from purified γ-casein. It is suggested that the locus symbol γ-Cn be assigned for use in designating the γ-casein polymorphism. The codominant alleles may be designated $\gamma\text{-Cn}^A$ and $\gamma\text{-Cn}^B$, with the further subdivision of the A-type into $\gamma\text{-Cn}^{A1}$, $\gamma\text{-Cn}^{A2}$, and $\gamma\text{-Cn}^{A3}$, even though these are not independent of the β-casein alleles.

Whey Proteins

α-Lactalbumin. Recently, the protein α-lactalbumin has come under intensive study relative to the discovery of a biological role for it in the enzymatic synthesis of lactose. Alpha-lactalbumin has long been known as a major component of bovine milk whey and its counterpart has been isolated from the milk of many other spe-

cies. The enzyme lactose synthetase[3] catalyzes the following reaction as the last step in the enzymatic synthesis of lactose (32):

Uridine Diphosphate Galactose (UDPGal) + Glucose ⟶ Lactose + UDP

Brodbeck and Ebner (17, 18) found that the lactose synthetase activity present in the mammary gland and milk of rats and cows was dependent on the presence of two proteins; these two protein components were named A and B protein subunits of lactose synthetase. High concentrations of the B protein subunit were found in milk and this protein was subsequently isolated in the crystalline state from bovine milk and shown to be identical to the protein α-lactalbumin (16).

Studies with rat and bovine mammary tissue (8, 17, 18, 27) have indicated that the A protein subunit is bound chiefly to the cellular particulate matter; as yet it has not been isolated in a pure state. The A protein subunit was further identified by Brew et al. (15) as a more general galactosyltransferase found normally in a variety of tissues. In the absence of the B protein subunit, the A protein subunit was found (15) to transfer enzymatically the galactose from UDPGalactose to a number of compounds such as N-acetyl glucosamine but not to glucose itself, unless it is present in very high concentrations (31). Found in mammary tissue before lactation, in the presence of the B protein subunit (α-lactalbumin) present during lactation, and dependent on its concentration, the A protein subunit now transfers galactose from UDPGalactose to glucose to make lactose. No catalytic function has been found for the B protein subunit alone and, thus, Brew et al. (15) have called it a "specifier" protein.

Proteins identified as α-lactalbumins have been isolated from the milks of various species including the human (81, 123), goat, pig, sheep, and rat (123), and the guinea pig (13) in addition to the cow. Ebner et al. (33, 123) have found that the α-lactalbumins isolated from these species all have activity as the B protein subunit when used with purified bovine A protein subunit. It should be noted that not all of these α-lactalbumins have similar physical, chemical, or immunological properties, even though they have a similar enzymatic function. The α-lactalbumins of the ruminant species have been shown previously by Johke et al. (62) to be very similar immunologically to each other; however, collectively they bear little immunological relationship to the B protein subunits present in the milk of other species.

Further work by Brew et al. (14) has shown that the B protein subunit (α-lactalbumin) of bovine milk has an amino acid composition and sequence very similar to that of the enzyme lysozyme isolated from the white of the hen's egg, and preliminary studies on the three-dimensional structures have indicated a close relationship, suggesting a very similar genetic evolutionary background for these two proteins (104). Notwithstanding the similarities in structure, the enzymatic functions of these two proteins are distinct, and lysozyme will not substitute for α-lactalbumin in the lactose synthetase complex nor does α-lactalbumin have any activity as lysozyme. The amino acid sequence and three-dimensional structure of the lysozyme found in bovine milk in low concentration is not yet known.

This role of α-lactalbumin in the enzymatic synthesis of lactose is a very interesting control mechanism whereby the activity of an enzyme already present is redirected to make a product specific to lactation. Enzyme complexes or sub-

[3] The systematic name under the International Union of Biochemistry, Commission on Enzymes, is: UDP-galactose-*d*-glucose 1-galactosyltransferase (EC 2.4.1.22).

TABLE 4. Amino acid composition of various γ-caseins.

Amino Acid	Residue Numbers		
	—A[2] [a]	—B[a]	[b]
Aspartic Acid	9	9	8
Threonine	10	10	9
Serine	13	12	13
Glutamic Acid	39	39	39
Proline	41	40	37
Glycine	5	5	5
Alanine	6	6	6
Valine	20	20	22
Methionine	7	7	7
Isoleucine	8	8	8
Leucine	23	23	23
Tyrosine	5	5	5
Phenylalanine	11	11	9
Tryptophan	1	1	1
Lysine	12	12	11
Histidine	6	7	6
Arginine	3	4	3
Phosphate	1	1	1

[a] Taken from Groves and Gordon (46).

[b] Calculated on the basis of the data of Gordon et al. (42), assuming mole wt = 25,000.

units are known in bacterial systems where two proteins may also be required for catalytic activity, especially those enzymes of a regulatory nature where one subunit directs in an allosteric manner the functioning of the active site of the enzyme present in the other protein (32). However, this is the first such system to be described from a mammalian source. In comparison with the bacterial systems, it is unusual to have one of the subunits present in very high concentration and to find the other or active subunit performing a different function in its absence.

The recent article of Ebner and Brodbeck (32) may be consulted for an excellent review of the biological role of α-lactalbumin.

The Committee recommends at this time that both the terminology "α-lactalbumin" and the "B protein subunit of lactose synthetase" be retained for this protein. In reporting studies, care should be taken to designate the species from which such a protein is isolated. In the future, protein isolated from the milk of some species would be appropriately named as an α-lactalbumin, if it functions as the B protein subunit in the synthesis of lactose, but would be given some other name if it does not perform this function, even though it may have physical, chemical, structural, or other properties similar to the α-lactalbumin of bovine milk. Thus, the Committee believes that the terms α-lactalbumin and the B protein subunit of lactose synthetase can be used in a synonymous sense with each other.

We realize that there are objections to this recommendation for the retention of a trivial name of a protein which has been implicated as a subunit in an enzymatic function. However, the Commission on Enzymes of the International Union of Biochemistry[3] will have difficulty with the proper nomenclature of the subunits until the composition of the complex between the A and B proteins is known. Although not probable, it is also conceivable that some additional biological function might be found for α-lactalbumin.

β-Lactoglobulin. At the time of preparation of the last Revision (126), three variants of β-lactoglobulin had been reported and their amino differences determined. Shortly thereafter, a fourth or D variant was reported (44) and its occurrence has been confirmed (75, 85, 86), but amino acid analyses are not currently available. Additional variants from Australian Droughtmaster cattle have also been reported (10, 82); these are believed to be alleles of the A and B variants, so have been designated A_{Dr} and B_{Dr} (10). McKenzie (82) reported that A_{Dr} contained carbohydrate, but the significance of this observation relative to the nomenclature cannot be assessed at present.

Immunoglobulins[4]

This section attempts to describe the general characteristics and nomenclature of immunoglobulins, to summarize the types of bovine immunoglobulins in milk, and to introduce a nomenclature consistent with that used for more extensively studied species. Extensive reviews of the immunoglobulins of other species (28, 66, 99) and of the cow (22) can be found elsewhere.

Characteristics of immunoglobulins. The term immunoglobulin is general and applies to a heterogeneous family of large molecular weight proteins that share common physico-chemical characteristics and antigenic determinants. These proteins occur in serum and other body fluids, exhibit γ- or slow β-electrophoretic mobility, and include all molecules with antibody activity. The term immunoglobulin replaces terms like "immune lactoglobulin," "γ-globulin," "euglobulin," "pseudoglobulin," and "T-globulin" that can be found in earlier dairy science literature.

The family of immunoglobulin molecules have related structures. All immunoglobulins appear to be either monomers or polymers of a four-chain molecule consisting of two light polypeptide chains (L-chains:20,000 mole wt) and two heavy polypeptide chains (H-chains), with molecular weights varying from 50,000 to 70,000 for the different immunoglobulin classes (Fig. 3). Immunoglobulin IgM is a pentamer of the four-chain unit, IgA is often a dimer, and IgG is normally a monomer. Immunoglobulin structure is normally studied by reduction and alkylation of intact molecules to yield their constituent polypeptide chains. In addition, immunoglobulins can be fragmented by proteolytic enzymes or cyanogen bromide. Digestion (Fig. 3) with papain yields two Fab fragments, composed of one light chain and half of one heavy chain; and a single Fc fragment, composed of the remaining portions of the two disulfide-linked heavy chains. The Fc portion of the molecule contains the carbohydrate moiety and the half-cystine residues which bind the subunits of the IgM and IgA polymers. The carbohydrate content is relatively low for

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[4] Contributed by J. E. Butler, Eastern Utilization Research and Development Division, USDA, Washington, D.C. 20250.

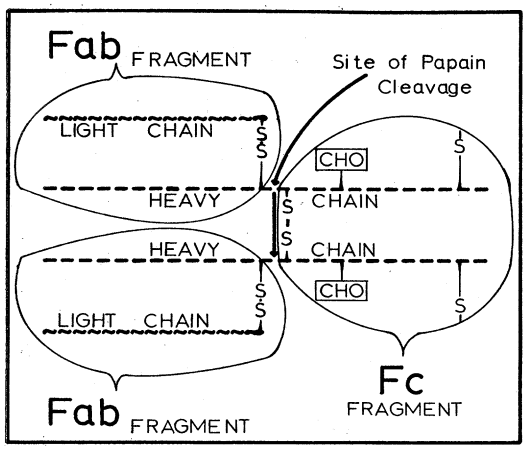

FIG. 3. Classical four-polypeptide-chain structural unit of an immunoglobulin. Heavy and light chains, site of papain cleavage, and digestion fragments are labelled. The half-cystine residues shown on the right end of the heavy chains bind the IgA and IgM subunits. CHO = Carbohydrate moiety.

IgG (2 to 3%) and higher for all others ($\simeq$10%).

It should be emphasized that immunoglobulins were historically, and are currently, separated into classes on the basis of their antigenic determinants. Immunoelectrophoresis is the technique normally employed for the initial identification of these molecules. Modern investigations have shown that specific physico-chemical features of the molecules of each class are responsible for these antigenic differences. Because the light polypeptide chains are common to the immunoglobulins of all classes, the antigenic and physico-chemical differences reside in the heavy polypeptide chains. Hence, each immunoglobulin class has distinctive heavy chains which are named γ- for IgG, μ- for IgM, α- for IgA, δ- for IgD, and ϵ- for IgE. Smaller antigenic and physico-chemical differences among the molecules within a class give rise to subclass designations. In man, the best-known species, the five classes have been identified, with IgG represented by four subclasses and IgA by two subclasses.

Antigenic and physico-chemical differences among classes and subclasses of immunoglobulins are also correlated with differences in biological activity. The functional aspects of bovine immunoglobulins will be treated elsewhere (22).

Bovine lacteal immunoglobulins. Three antigenically distinct classes of bovine immunoglobulins have been reported. All occur in the lacteal secretions and serum and are designated IgM (γM), IgA (γA), and IgG (γG). The IgG class is divided into two subclasses: IgG1 (γ1) and IgG2 (γ2). The evidence supporting this classification will be discussed in a forthcoming review (22). Although both the Arabic and Greek letter designations are in accord with the World Health Organization nomenclature report (20), the Arabic will be used throughout this section of the revision. The structure and occurrence of immunoglobulins in the lacteal secretions of mammals deviates from the pattern which is characteristic for such proteins in the sera of these organisms. At least one such deviation has long been recognized in the cow. This concerns the selective accumulation of bovine IgG1 in the colostrum and normal milk. A summary of each class of bovine lacteal immunoglobulins follows.

Bovine IgM. A macroglobulin having the same physico-chemical and biological properties as the IgM of other species has been isolated from colostral and normal whey (21, 43, 60, 91). In addition, immunoelectrophoretic precipitin arcs characteristic for an IgM immunoglobulin have been demonstrated (Fig. 4) (9, 51, 53, 54, 108, 110). This immunoglobulin has a sedimentation constant of 19 S, is sensitive to 2-mercaptoethanol, and has been reported to contain 12.3% carbohydrate (43). When analyzed by disc electrophoresis at pH 4.3, IgM does not enter the separating gel but forms a dense band at the separating gel/stacking gel interface. IgM is eluted in the first peak (void volume) from a Sephadex G-200 fractionation when a fraction of whey insoluble in 33% $(NH_4)_2SO_4$ is used as the starting material. Alpha-2-macroglobulin can be removed from the IgM preparation by Pevikon block electrophoresis (69 92).

Bovine IgG1 and IgG2. The most abundant immunoglobulins of milk belong to the class IgG. All contain 2 to 4% carbohydrate (43, 45, 64, 97, 118) and sediment as approximately 7 S molecules upon ultra-centrifugation, although a 19 S IgG has been reported (52). The group can be subdivided into subclasses by immunodiffusion, immunoelectrophoresis, anion-exchange chromatography, electrophoresis, and ethanol-fractionation (21, 45, 56, 64, 68, 91, 93, 116). The more basic IgG molecules are called the IgG2 immunoglobulins and have a mean S_{20w} value of 6.6. These molecules move most rapidly to the cathode during agar electrophoresis at pH 8.2[5] and acrylamide gel electrophoresis at pH 4.3. The IgG2 immunoglobulins are not retained on DEAE-cellulose at low ionic

[5] Moves cathodally as a result of electro-osmosis.

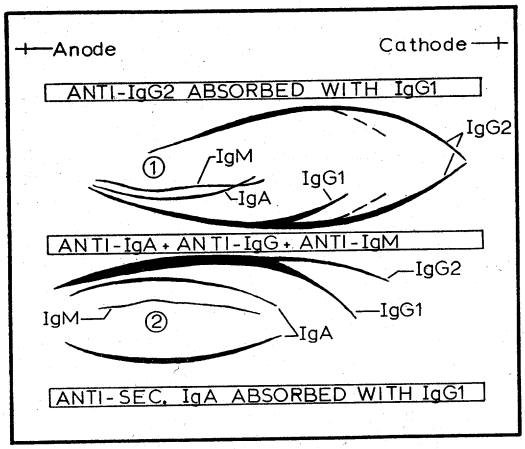

FIG. 4. Diagram of generalized immunoelectrophoretic pattern of bovine serum and lacteal immunoglobulins (21). Well 1 contains normal bovine serum, Well 2 contains colostral or normal whey. Antisera in troughs are labelled. Sec. IgA = Secretory IgA. Dotted arc indicates position of Kickhöfen's γ1 and Grove's colostral IgG2. Modified from Haurowitz (55).

strength, pH 8.3, and hence are eluted in the break-through peak. Although abundant in serum, the concentration of IgG2 is very low in the lacteal secretions (9, 106, 127) and may be actually decreased during colostrum formation (25, 26) (Fig. 4).

The subclass IgG1 consists of the less basic IgG immunoglobulins, which are eluted at higher ionic strength than IgG2 during fractionation of whey on DEAE-columns and which appear more heterogeneous on immunoelectrophoresis and ion-exchange chromatography than IgG2 (21). The mean S_{20w} value for the IgG1 subclass is 6.3, although considerable deviations from this value have been reported. IgG1 is the principal immunoglobulin of the lacteal secretions, especially in colostrum (25, 26, 88, 91, 106, 110, 116, 119) (Fig. 4). As much as 50 to 75% of the colostral protein in the cow is composed of immunoglobulins, nearly all of which is IgG1 that has been selectively transported from the serum (11, 25, 30, 38, 39, 76, 78). The lacteal IgG1 molecules are identical to those of the serum (51, 90, 97, 105, 106), although there is some evidence that a small change may occur during transport (64).

The two subclasses differ antigenically (21, 64, 106) and in amino acid composition (45, 64, 89). The IgG1 molecules have a lower basic amino acid content (45) and higher half-cystine content than IgG2 (64). The subclasses appear to share a common Fab fragment (93), but differ antigenically in their Fc fragments (64, 115). The antigenic (37), electrophoretic (37, 64), and amino acid composition and sequence (89) differences between isolated γ-chains of IgG1 and IgG2 may reside in their Fc fragments.

In a recent paper by Kickhöfen et al. (64), the IgG immunoglobulins were subdivided into three subgroups on the basis of their behavior on DEAE-Sephadex and immunoelectrophoresis. These investigators refer to IgG1 as γGs (secretory γG), IgG2 as γ2, and an intermediate subgroup as γ1. The γ1 and γ2 subgroups differ in charge but could not be distinguished immunologically, by half-cystine content, or by molecular weight. Kickhöfen et al. (64) reported a molecular weight of 163,000 for γGs (IgG1) and 150,000 for γ1 and γ2 (IgG2), both of which conflict with the average S_{20w} value for the IgG1 and IgG2 reported in this revision. Originally, Pierce and Feinstein (106) reported three IgG subgroups, but have more recently classified their intermediate component as IgG1 (89). Groves (45) has isolated an IgG immunoglobulin from colostrum which resembles the γ1 of Kickhöfen. Recent studies (21) suggest that it, like Kickhöfen's γ1, should be considered an IgG2 immunoglobulin. Nevertheless, such reports indicate that division of the IgG immunoglobulins into only two subclasses may be an oversimplification.

The two subclasses of IgG can be correlated with the early preparations of Emil Smith (119) in the following manner (21). Although antigenically heterogeneous, Smith's pseudoglobulin and plasma T-globulin contain mostly IgG1. The pseudoglobulin fractions also contain "secretory IgA" (see later). Smith's serum γ-globulin contains both IgG1 and IgG2 (mostly IgG2), and his euglobulin consists of IgG2-like globulins, slower IgG1 globulins, IgA, and IgM.

Bovine IgA. An antigenically distinct immunoglobulin with slightly different ion-exchange behavior than bovine IgG1 has been reported in the lacteal secretions (9, 24, 43, 54, 60, 91). Because of the lack of collaboration among investigators, it is possible that each has reported a distinct but different immunoglobulin. From the data available, this possibility seems remote and the immunoglobulin in all cases is probably IgA. On the contrary, the immunoglobulin designated IgA by some (3, 9) is most certainly IgG1.

The IgA immunoglobulin isolated from the milk is sensitive to 2-mercaptoethanol (24, 60), has a carbohydrate content of 8 to 9% (43), and a sedimentation coefficient of 10 to 12 S (24, 60). Lacteal IgA is eluted between the

IgM and IgG peaks during Sephadex G-200 fractionation of whey. It has been demonstrated that glycoprotein-a (45) occurs both free and bound to lacteal IgA (23). Hence, glycoprotein-a and lacteal IgA are probably respectively homologous to the "secretory (transport) piece" and "secretory IgA" described for other species.

The 10 S contaminant in early preparations of pseudoglobulin (45, 56, 103, 117) may have been secretory IgA or aggregated IgG1.

Proteose-Peptone Fraction

McKenzie (82) has suggested that the term "proteose-peptone" be dropped, and we agree that in the light of present knowledge (Table 5) the term is inaccurate and unsuitable. However, present knowledge does not permit us with assurance to assign the components of this mixture to their proper categories; therefore, we have retained the historic proteose-peptone grouping for the present.

The proteose-peptone fraction is conveniently defined as that portion of the protein system not precipitated by heating at 95 to 100 C for 20 min and subsequent acidification to pH 4.7, but precipitated by 12% (w/v) trichloroacetic acid (111). The proteose-peptone proteins account for 18 to 25% of the serum proteins and about 4% of the total proteins in milk. This fraction exhibited three electrophoretically discernible peaks in a moving-boundary Tiselius cell and these were designated milk serum Components "3," "5," and "8" in ascending order of electrophoretic mobility (77). Zonal electrophoresis in polyacrylamide gels, employing a continuous borate buffer system, revealed a greater degree of heterogeneity (71). When stained with Amido Black, Component "3" showed a single zone, Component "5" appeared as a close-migrating doublet, whereas Component "8" separated into two, multi-zonal areas. When developed for glycoproteins, additional zones were detected, especially in the area of the gel occupied by Component "3." The two staining procedures revealed approximately 15 electrophoretically discernible zones.

Preparative gel electrophoresis, gel filtration, addition of ammonium sulfate in combination with pH adjustments were employed singularly or in combinations to prepare the four principal fractions designated as serum Components "3," "5," "8-slow," and "8-fast" (71, 96). Selected compositional and physical characteristics of these components are listed in Table 5. These data indicate that the proteose-peptone system is composed primarily of low-molecular weight glycoproteins. Their amino acid compositions are characterized by low concentrations of the aromatic residues and relatively high concentrations of glutamic and aspartic acids. Low concentrations of methionine accounted for

TABLE 5. Compositional and physical properties of proteose-peptone fractions.

Constituent (%)	Component "3"	Component "5"	Component "8-slow"	Component "8-fast"
Nitrogen	13.1	13.8	12.3	13.3
Phosphorus	0.5	1.0	2.0	2.4
Hexose	7.2	0.9	4.5	1.4
Hexosamine	6.0	0.2	2.5	0.3
Sialic Acid	3.0	0.3	3.3	0.4
Property				
Electrophoretic Mobility[a] ($\times 10^{-5}$ cm^2 v^{-1} sec^{-1})	−3.8	−4.8	−9.2	−9.3
Isoelectric point (pH)	3.7	....	....	3.3[b]
$S^{\circ}_{20,w}$ ($\times 10^{-13}$g)[c]	4.0	1.2	1.4	0.8
$\overline{M}^{\circ}_{w}$[d]	200×10^3	14.3×10^3	9.9×10^3	4.1×10^3
	40×10^3[e]	.....	.....	.....
$D^{\circ}_{20,w}$ ($\times 10^{-7}$ cm^2 sec^{-1})[c]	1.8	.....	.....	.....

[a] Average of descending and ascending channels in veronal buffer, pH 8.6, $\Gamma/2 = 0.1$.
[b] Component "8" (mixture of "8-slow" and "8-fast").
[c] Determined in veronal buffer, pH 8.6, $\Gamma/2 = 0.1$.
[d] Sedimentation-equilibrium at infinite dilution in veronal buffer, pH 8.6, $\Gamma/2 = 0.1$.
[e] $\overline{M}^{\circ}_{w}$ Determined in presence of 5 M guanidine-HCl.

the sulfur-containing residues. Component "5" contained 10.6% proline. The carbohydrate moiety consisted of galactosamine, glucosamine, galactose, glucose, mannose, fucose, and sialic acid.

Component "3" was identified as a serum protein, whereas Fractions "5" and "8" were found in the serum as well as in casein micelles, from which they seem to originate. All three components have been isolated from both heated and unheated skimmilk, thus refuting the idea that they represent heat-induced artifacts.

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